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Normalization time window of recombinant human endostatin: an overview

Lang He

Department of Oncology, Chengdu Fifth People's Hospital, The Second Clinical Medical School of Chengdu University of Traditional Chinese Medicine, Chengdu, 611130, Sichuan, China

Abstract: **Anti-angiogenic therapy has emerged as the research frontier of cancer treatment with the concept of starving tumor cells by suppressing tumor neovascularization. It is well known that tumor blood vessels are different from regular blood vessels in terms of structure and function. With the advance of anti-angiogenic research, it was discovered that anti-angiogenic agents could transiently normalize tumor blood vessels to be structurally and functionally similar to regular blood vessels. This short period is called normalization time window of tumor vasculature. Accumulating evidence supports that administering anti-angiogenic drugs combined with radiotherapy or chemotherapy in the normalization time window enhances efficacy of cancer therapeutics. Recombinant human endostatin (rh-ES) is a novel anti-angiogenic agent with complete independent intellectual property rights in China. It is of vital importance to determine the normalization time window of rh-ES to guide clinical planning and treatment in the future. The recent progress in normalization time window of rh-ES was comprehensively reviewed.**

Keywords: Recombinant human endostatin; Time window; Anti-angiogenic therapy; Vasculature normalization; Targeted therapy

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*Corresponding Author: Lang He, helang729@163.com

1. Introduction

Traditional chemotherapy remains the main therapeutic strategy for cancer, but it seems to have reached a plateau, especially for the treatment of lung cancer[1]. With the discovery of cancer driver genes and immune checkpoint and their subsequent successful implementation in clinic.Targeted therapy and immunotherapy have been highlighted due to their superior therapeutic efficacy over conventional chemotherapy, especially for patients with known driver genes[2-4]. The treatment of malignant tumors is gradually moving towards the era of targeted therapy and immunotherapy. In the context of targeted molecular therapy, biomarkers under standardized settings are used to define a specific gene or gene profile that favor tumor growth and personalized therapy based on a patient's unique gene expression profile is a promising approach to specifically target the tumor. As one of the research hotspots in the field of cancer treatment, molecular-targeted drugs have the advantages of accurate position, strong pertinence, and low toxicity. Anti-angiogenic therapy, as a critical component of targeted molecular therapy, has been widely used in clinical practice and achieved desirable outcomes in treatment of various types of cancers[5].

The anti-angiogenic therapeutic strategy was initially proposed based on Folkman's hypothesis in the 1970s[6] that, since tumor neovascularization is essential to tumor growth and metastasis, control of tumor growth can be achieved by inhibiting formation of new blood vessels that supply the tumor with nutrition. Accumulating evidence supports the crucial role of neovascularization in promoting tumor growth, invasion, and metastasis. Therefore, it was reasonable to expect that the use of anti- angiogenic drugs could lead to tumor angiogenesis inhibition, tumor vascular regression, ischemia and hypoxia of tumor tissue, and eventually tumor necrosis. However, this expectation was not achieved in subsequent anti-angiogenic therapy studies. Instead, there is growing evidence supporting the hypothesis that reducing the hypoxic environment in tumors and improving vascular delivery can enhance anti-tumor effects induced by conventional chemotherapy and radiotherapy. Based on this perspective, a new theory so called vascular normalization theory emerged to explain these researching findings. Currently, anti-angiogenic agents that are used in clinic as effective drugs for cancers include bevacizumab, sorafenib, sunitinib, pazopanib, recombinant human endostatin (rh-ES) and so on[7,8]. Here, we reviewed the research on the vascular normalization time window of rh-ES.

2. Rh-ES and mechanism of action

Rh-ES is a novel vascular endothelial inhibitor that was developed independently by Chinese scientists with complete independent intellectual property rights[9]. Constructed through recombining the N-terminal domain of endostatin with a nine-amino-acid sequence (MGGSHHHHH) and expressed in Escherichia coli as a host expression vector, rh-ES exhibits longer half-life, prolonged

stability and enhanced biological activity[10,11]. In 2005, rh-ES was approved by China Food and Drug Administration (CFDA) combined with vinorelbine and cisplatin for both initial and secondary treatment of patients with advanced non-small cell lung cancer (NSCLC) and was included in the National Comprehensive Cancer Network (NCCN) of United States treatment guidelines (Chinese Version)[12].

Rh-ES is a board-spectrum anti-angiogenic agent that exerts its effects by involving in multiple intracellular signaling pathways, including the vascular endothelial growth factor/vascular endothelial growth factor receptor 2 (VEGF/VEGFR-2), Wnt/β-catenin, and Src signaling transduction cascades, among which the most important is the VEGF signaling pathway. Rh-ES can directly inhibit the phosphorylation of KDR/Flk-1 (VEGFR-2) thereby reducing the total protein levels of VEGFR-2, and indirectly limit activation of extracellular signal-regulated kinase (ERK), p38 mitogen-activated protein kinases (p38 MAPK), and AKT. Suppression of these signaling molecules can inhibit neovascularization by inhibiting osteopontin (OPN)-induced vascular endothelial cell migration and proliferation, and meanwhile promote apoptosis of human umbilical vein endothelial cells (HUVECs) by activating intracellular Caspase-3 and inactivating B-cell leukemia/lymphoma 2 (Bcl-2)[13-16]. Rh-ES can induce tumor vascular normalization via p38 phosphorylation and inhibition of anterior gradient 2 (ARG-2) in HUVECs-medicated activation of matrix metalloproteinase-2 (MMP-2), cMYC, Ve-cadherin, and ERK 1/2[17]. Additionally, rh-ES can block OPN-mediated signaling pathways via inhibiting the production of MMPs and eventually diminish tumor invasion and tumor vasculature[16,18,19]. Rh-ES may target cytoplasmic and nuclear β-catenin or its downstream effectors directly to reduce their availability, which leads to suppression of the Wnt/β-catenin signaling pathway and reduction in cyclin D1 and VEGF proteins, and eventually decrease in angiogenesis[20]. Besides, rh-ES has been shown to inhibit tumor growth accompanied with decreased level of serum basic fibroblast growth factor (b-FGF). Moreover, Rh-ES could attenuate b-FGF-activated phosphorylation of p38 and ERK1/2 in HUVECs and reduce new vessel formation[21]. Rh-ES has demonstrated its anti-angiogenic effects in a mouse model of non-Hodgkin's lymphoma, in which rh-ES inhibited the proliferation of endothelial progenitor cells by suppressing the production of p-AKT (non-p-ERK)[22]. Furthermore, under low oxygen conditions (hypoxia, 1% oxygen), rh-ES can overcome oxaliplatin resistance via inhibiting the hypoxia-inducible factor 2α/CXC chemokine receptor 4 (HIF-2α/CXCR4) signaling pathway[23]. It should be noted that these anti-angiogenic effects of rh-ES are dose-dependent. Radiotherapy is vital in cancer treatment. Accumulating studies indicated that rh-ES combined with radiotherapy has the potential to enhance radiation sensitivity via inhibiting the expression of TGF-β1, HIF-1α, MMP-2 and VEGF, both directly and indirectly[24-26]. The AKT signaling pathway was also found to be involved in rh-ES-mediated radiation sensitivity, including inhibition of p38 MAPK and Akt phosphorylation, suppression of Bcl-2 protein expression, and activation of caspase proteins[27]. Notably, although rh-ES is a board-spectrum anti-angiogenic agent, the anti-angiogenic activities of rh-ES are selective for undifferentiated blood vessel in tumor tissues[28].

3. Anti-angiogenic therapy time window: concept and background

After the anti-angiogenic therapeutic strategy was formulated, anti-angiogenic therapy has attracted wide spread interest and has been extensively studies. Although this therapy has demonstrated anti-tumor activity in clinical practice, its therapeutic efficacy is not as excellent as expected. Extensive research has suggested that excessive inhibition of angiogenesis could hinder the transport of therapeutic agents and oxygen to the targeted tumor tissue, and further aggravate tumor hypoxia and diminish efficacy of chemotherapy or radiotherapy. These findings are against the expectation that combination with anti-angiogenic agents as a way to improve efficacy of tumor radiotherapy and chemotherapy. In order to explain these paradoxical findings, Jain RK first hypothesized the concept of normalization of tumor vasculature. It was hypothesized that judicious application of antiangiogenic drugs can fix abnormal tumor vasculature before degradation occurs, normalize the structure and function of tumor blood vessels, thereby improve tumor microenvironment and render more efficient delivery of oxygen and drugs to the targeted tumor cells[29,30]. Subsequent preclinical and clinical studies supported this hypothesis, finding that anti-angiogenic therapy is capable to induce normalization of tumor vasculature and microenvironment[31]. The time period during which tumor vessels initially become normalized is defined as “normalization time window”. This normalization time window is a transient and reversible period during which increased sensitivity to radiation or chemotherapy is accompanied with functional changes characterized by reorganized disordered tumor blood vessels, thickened vascular basement membrane, increased perivascular cell coverage, improved tumor blood perfusion, reduced vascular permeability, and increased partial pressure of oxygen[29,32,33]. This normalization of tumor vasculature is a complex process with multiple determinants, such as dose and type of anti-angiogenic drugs, delivery vehicles of the drugs, and tumor types and locations[17,34-36].

According to Jain's hypothesis[29,30], the most efficient strategy is to administer radiotherapy and chemotherapy combined with anti-angiogenic drugs in this period of normalization window. As such, determining the normalization time window of various anti-angiogenic agents is of vital importance to optimize combined therapy strategies with anti-angiogenic agents and maximize clinical outcomes.

4. Normalization time window of tumor vasculature induced by rh-ES

4.1. Basic research

4.1.1 Experimental methods of normalization time window

To determine the time window for tumor vessel normalization, a common approach is to monitor specific biomarkers that timely reflect structural and functional changes of the vessels occurring during the transaction process of tumor vascular normalization. Temporal changes of these biomarkers are used to indicate the normalization time window. Various biomarkers have been studied as candidates for monitoring the vascular normalization, including Ang1/Ang2 and Tie2 for vascular stability, PHD2 for cellular oxygen sensing, regulator of G protein signaling 5 (RGS5) for vascular maturity, CD31 and CD105 for endothelial cell neovascularization, α-smooth muscle actin (α-SMA) and collagen IV for microvascular maturity, and HIFs for hypoxia[19,33,37-40]. Imaging techniques such as laser confocal microscopy and contrast ultrasound are a potential more direct and effective way to determine normalization time window by measuring the changes in microvessel density (MVD), blood perfusion, and vascular permeability. Besides, high performance liquid chromatography (HPLC) has been proven to be a powerful method to detect tissue-level changes of drug concentration, which indirectly indicate the time period of tumor vascular normalization[41]. Recent advances in computed tomography (CT) perfusion imaging, functional magnetic resonance imaging (MRI) and positron emission tomography-computed tomography (PET/CT) have opened opportunities to visualize the normalized morphology of blood vessels in a more convenient, reliable and efficient way.

4.1.2 studies of vascular normalization time window by rh-ES

Researchers have used different techniques and approaches to establish the vascular normalization time window induced by rh-ES and produced different results. Xin et al. and He et al. found that at days 4 and 6 after treated with rh-ES in a SKOV3 ovarian cancer mouse model (20 mg/kg, subcutaneously, once a day) and in a Lewis lung cancer mouse model (5 mg/kg, intraperitoneal, once a day), respectively, α-SMA and collagen IV expression levels were significantly increased while expression of CD31, RGS5, and HIF-1α were significantly decreased, indicating that rh-ES created a vascular normalization window characterized with increased numbers of microvascular endothelial progenitor cells, thickened basement membrane, normalized vascular structure, and improved oxygen status as well as microvascular permeability[39,42]. Further evaluation of rh-ES combined with cisplatin demonstrated the tumor growth inhibition effect in the xenograft model of ovarian cancer, and confirmed the normalization window of days 4-6 after rh-ES administration[39]. Huang and Chen also employed the established xenograft nude mouse model of Lewis lung cancer and treated the mice with subcutaneous injection of rh-ES (5 mg/kg, once a day)[43] . They observed that 7 days after rh-ES administration, collagen covered tumor microvessel and perivascular coverage around endothelial cells were significantly increased, along with transient normalization of tumor blood vessels. In addition, on days 3-5 and 6-8, the anti-tumor effect of paclitaxel was significantly strengthened by rh-ES. In a xenograft mouse model of lung adenocarcinoma A549, it was revealed that 4 days after rh-ES treatment, expression of extracellular MMP inducer, VEGF, MMP-2 and MMP-9 were decreased and 7 days after rh-ES treatment, tumor vasculature was normalized with decreased MVD, vessel permeability and intratumoral hypoxia, and increased collagen coverage[44]. Given these results, it was generally believed that the vascular normalization window was days 4-10 after rh-ES administration. However, the results of rh-ES combined therapy with cisplatin were a little different, showing that the maximum anti-tumor effect occurred 5-9 days after rh-ES administration[44]. Yu et al. found that rh-ES induced tumor vascular normalization via up-regulation of SRCIN1 protein in the vascular endothelial cells to inhibit the Src signaling pathway; the vascular normalization window was from day 6, when drugs penetrated tumor tissue more efficiently[45]. Xu et al. assessed changes of biomarker levels in a nude mouse model of hepatocellular carcinoma (HCC) and found that the vascular normalization window by rh-ES was days 3-9. They argued for involvement of immune response as rh-ES was found to increase the levels of immune factors IFN-γ and CD 86 while reduce the IL-17 level in liver tissue of the HCC mice. It was speculated that normalization of tumor vasculature was correlated with inhibition of Th17 and regulation of Th1/Th2 imbalance[46]. Will the results be the same if rh-ES is administered in combination with radiotherapy? In the mouse models bearing CNE-2 and 5-8F human nasopharyngeal carcinoma xenografts, Peng et al. found that the normalization time windows of tumor vasculature were days 5-7 and days 3-5, respectively,

after induction with rh-ES (20 mg/kg, subcutaneously, once a day). Within the normalization time window, both mouse models exhibited down-regulation of CD31, VEGF, MMP-2, MMP-9, MMP-14, up-regulation of pigment epithelium-derived factor (PEDF), increased perivascular cell coverage, decreased proportion of hypoxic cells, and decreased number of disordered blood vessels. The maximum anti-tumor effect occurred on day 5 and day 3, respectively, after rh-ES administered concurrently with radiotherapy[19]. In a nude mouse model bearing ECA109 esophageal squamous cell carcinoma (ESCC) xenograft, Zhu et al. observed normalized tumor vasculature in the transplanted tumor 5 days after rh-ES use (15 mg/kg, intravenously, once a day). Combined radiotherapy at this period produced the most significant inhibition of tumor growth, corresponding to reduced MVD, increased number of pericytes and endothelial cell coverage, and improved hypoxic environment in the tumor. It was suggested that rh-ES improved response to radiotherapy in the ECA109 ESCC cells by regulating the HIF/VEGF pathway and reducing tumor vascular remodeling and hypoxia[12]. Zheng et al. took a different approach by monitoring changes of lactic acid, lactate dehydrogenase mRNA, and pH value in tumor microenvironment over multiple time points in the nude mouse model of Lewis lung cancer treated with rh-ES combined with radiotherapy. They found significantly reduced lactic acid and lactate dehydrogenase mRNA, along with increased pH in tumor microenvironment from acidic to alkaline and improved tumor hypoxia on days 6-10 after rh-ES treatment. Based on these results, the researchers argued that rh-ES could increase the radiation sensitivity of tumor through inhibiting glycolysis and reducing lactic acid production in the tumor cells[47]. Drug delivery vehicles are a known factor to influence drug pharmacodynamics. It is of great research interest to known whether increased targeting ability enhance drug efficacy. Li et al. employed gold nanoparticles to advance the transport capacity and transport rate of rh-ES to the targeted tumor site. Their study showed that on days 4-8 of rh-ES administration, the tumor blood vessels were transiently normalized, accompanied with obviously increased blood flow and 5-fluorouracil concentration in the tumor tissue[48]. Molecular biomarkers and functional imaging are increasingly shown to have prediction value of anti-angiogenic therapy effect, but their strengths are different. Biomarkers are able to predict drug response via monitoring changes in vascular endothelial cells while functional imaging can be used to directly monitor numbers of tumor cells, both of which are important to guide rational use and appropriate timing for drugs[49]. For example, Jia et al. applied both molecular biomarkers and bioluminescence imaging to evaluate response to anti-tumor therapy through a mouse model of NUGC-4 gastric cancer with peritoneal metastasis. They concluded that the concurrent use of rh-ES and cisplatin had superior outcomes in terms of killing tumor cells and anti-angiogenesis than other sequential therapies, including cisplatin (days 1-3) plus rh-ES (days 4-7) and rh-ES (days 1-4) plus cisplatin (days 5-7). Furthermore, they found that the cisplatin-rh-ES sequential treatment was more effective in killing tumor cells than the rh-ES-cisplatin sequential treatment, while the latter had better anti-angiogenic effect[49].

Taken together, various preclinical studies have demonstrated that anti-angiogenic agents can lead to vascular normalization, but the real time of vascular normalization and optimal anti-tumor responses to combined administration of anti-angiogenic and chemo- and/or radio- therapy are not always within the "normalization time window"[50]. On the other hand, different sequential orders in drug combinations could lead to different therapeutic effects. Yuan et al. concluded that the optimal drug administration strategy should be simultaneous use of chemotherapeutic and anti-angiogenic drugs to shrink tumor size followed by anti-angiogenic drug alone to maintain remission induced by the combined therapy[51]. It should be noted that administration of anti-angiogenic drugs alone has limited therapeutic anti-tumor activity[33].

4.2 Clinical trials

Compared to the relative abundance of preclinical research data, clinical trials of normalization time window by rh-ES are limited and most were exploratory. Jiang et al. conducted a clinical study where patients with NSCLC were given rh-ES (7.5 mg/m^2) by intravenous drip for 10 days and evaluated with CT perfusion imagine and hypoxic imaging on days 1, 5, and 10. They found that in 10 days, tumor to normal tissue ratio (T/N) and capillary permeability surface (PS) decreased initially and then increased, reaching the lowest on day 5 (T/N, $P=0.00$, PS, $P<0.01$, compared with both days 1 and 10). Oppositely, blood flow (BF) increased initially and decreased afterward, reaching the highest on day 5 ($P<0.01$, compared with both days 1 and 10)[52]. These changes indicated that the normalization time window of rh-ES was around one week after use, consistent with some animal studies. To validate the normalization time window of rh-ES, Jiang et al. further applied a therapy strategy in which weekly rh-ES (15 mg/d) was administered by intravenous drip during the first week of radiotherapy for patients with NSCLC. Their results showed that in the rh-ES combined with radiotherapy and radiotherapy alone groups, the total effective rates were 80% and 44%, respectively; the difference was significant ($P<0.05$). In addition, the median progression survival rates were 21.1±0.97 and 16.5±0.95 months, respectively; the one-year and

two-year local control rates were 78.9% and 68.1% (P<0.05), 63.6% and 43.4% (P<0.05), respectively; the one-year and two-year overall survival rates were 83.3. % and 76.6% (P>0.05), 46.3% and 37.6% (P>0.05), respectively[50]. Meng et al. used rh-ES combined with radiotherapy to treat Lewis lung cancer-bearing mice and NSCLC patients and compared corresponding changes of hypoxia. Their results indicated that both in the mice and patients, the hypoxic environment was significantly improved 5 days after rh-ES use, and in the mice the radiotherapy-induced tumor growth inhibition effect was the most significant on this day[53]. Lv et al. conducted a clinical study of patients with advanced lung squamous cell carcinoma to evaluate the short-term effect of durative intravenous transfusion of rh-ES (30 mg/d) on days 1-7, combined with intra-arterial infusion with docetaxel and cisplatin on day 4 of vascular normalization window. Each cycle was repeated every 28 days and two cycles were delivered before evaluation. The efficacy evaluation showed that in the combined therapy group, the response rate and disease control rate were 70.0% and 90.0%, respectively, higher than in the pure arterial perfusion chemotherapy group (50.0% and 70.0%, respectively), but the differences were not significant (P=0.650 and P=0.582, respectively)[54].

Anti-angiogenic therapy has the advantage to normalize tumor vessels and overcome drug delivery barriers caused by abnormal tumor vasculature and hypoxia-induced chemoradiation resistance. Therefore, combined therapy guided by vascular normalization time window is proposed to improve anti-tumor treatment efficacy. Based on the abovementioned clinical results, compared with pure chemoradiotherapy, rh-ES combined therapy administered during the normalization time window is proven to provide better short-term clinical benefits, but the long-term survival benefits are largely unknown. This topic is worthy of further exploration and is likely the focus of future research.

5. Outlook and conclusion

Anti-angiogenic therapy is one of the most promising approach of targeted therapy for cancer, and its efficacy in combined with chemo- and/or radio-therapy is proven. The introduction of vascular normalization time window theory and subsequent research provide theoretical foundation and experimental basis for clinical guide and application of anti-angiogenic therapy to improve efficacy of cancer therapeutics. However, it remains largely unknown how to fully exert the effect or achieve best efficacy when anti-angiogenic agents applied in combined therapy. Although great progress has been made, there are certain confusions and challenges regarding the normalization time window of tumor vasculature: what is the golden standard for vascular normalization time window? How to effectively extend the normalization time window? Is this normalization time window applied to all types of tumors? Does its application influence long-term effect of tumor treatment? These are among many critical questions remain unanswered and deserve further research. Answering these questions will be the focus of future preclinical and clinical studies.

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References

[1] Ettinger DS, Wood DE, Akerley W, et al. NCCN guidelines insights: non-small cell lung cancer, version 4.2016[J]. J Natl Compr Canc Netw, 2016, 14(3):255-64.

[2] Afghahi A, Sledge G W, Jr. Targeted therapy for cancer in the genomic era[J]. Cancer J, 2015, 21(4): 294-8.

[3] La-Beck NM, Jean GW, Huynh C, et al. Immune checkpoint inhibitors: new insights and current place in cancer therapy[J]. Pharmacotherapy, 2015, 35(10):963-76.

[4] Marin-Acevedo JA, Dholaria B, Soyano AE, et al. Next generation of immune checkpoint therapy in cancer: new developments and challenges[J]. J Hematol Oncol, 2018, 11.

[5] Abusnina A, Keravis T, Zhou Q, et al. Tumour growth inhibition and anti-angiogenic effects using curcumin correspond to combined PDE2 and PDE4 inhibition[J]. Thromb Haemost, 2015, 113(2):319-28.

[6] Folkman J. Tumor angiogenesis: therapeutic implications[J]. N Engl J Med, 1971, 285(21):1182-6.

[7] Russo M, Giavazzi R. Anti-angiogenesis for cancer: Current status and prospects[J]. Thromb Res, 2018, 164 Suppl 1:S3-S6.

[8] Zhang K, Wang Y, Yu X, et al. Recombinant human endostatin combined with radiotherapy inhibits colorectal cancer growth[J]. BMC Cancer, 2017, 17(1):899.

[9] O'reilly MS, Boehm T, Shing Y, et al. Endostatin: an endogenous inhibitor of angiogenesis and tumor growth[J]. Cell, 1997, 88(2):277-285.

[10] Huang H, Liu Y, Lin QD, et al. Clinical observation of recombinant human endostatin in treating relapsed refractory multiple myeloma[J]. Clin Exp Pharmacol Physiol,

2018.
[11] Chen Y, Mathy NW, Lu H. The role of VEGF in the diagnosis and treatment of malignant pleural effusion in patients with nonsmall cell lung cancer (Review)[J]. Mol Med Rep, 2018, 17(6):8019-8030.
[12] Zhu H, Yang X, Ding Y, et al. Recombinant human endostatin enhances the radioresponse in esophageal squamous cell carcinoma by normalizing tumor vasculature and reducing hypoxia[J]. Sci Rep, 2015, 5:14503.
[13] Ling Y, Yang Y, Lu N, et al. Endostar, a novel recombinant human endostatin, exerts antiangiogenic effect via blocking VEGF-induced tyrosine phosphorylation of KDR/Flk-1 of endothelial cells[J]. Biochem Biophys Res Commun, 2007, 361(1):79-84.
[14] Kim YM, Hwang S, Kim YM, et al. Endostatin blocks vascular endothelial growth factor-mediated signaling via direct interaction with KDR/Flk-1[J]. J Biol Chem, 2002, 277(31): 27872-27879.
[15] Ling Y, Lu N, Gao Y, et al. Endostar induces apoptotic effects in HUVECs through activation of caspase-3 and decrease of Bcl-2[J]. Anticancer Res, 2009, 29(1): 411-417.
[16] Ni Q, Ji H, Zhao Z, et al. Endostar, a modified endostatin inhibits non small cell lung cancer cell in vitro invasion through osteopontin-related mechanism[J]. Eur J Pharmacol, 2009, 614(1-3):1-6.
[17] Pan F, Yang W, Li W, et al. Conjugation of gold nanoparticles and recombinant human endostatin modulates vascular normalization via interruption of anterior gradient 2–mediated angiogenesis[J]. Tumor Biology, 2017, 39(7): 101042831770854.
[18] Lu N, Ling Y, Gao Y, et al. Endostar suppresses invasion through downregulating the expression of matrix metalloproteinase-2/9 in MDA-MB-435 human breast cancer cells[J]. Exp Biol Med (Maywood), 2008, 233(8):1013-1020.
[19] Peng F, Xu Z, Wang J, et al. Recombinant human endostatin normalizes tumor vasculature and enhances radiation response in xenografted human nasopharyngeal carcinoma models[J]. PLoS One, 2012, 7(4): e34646.
[20] Xu X, Mao W, Chen Q, et al. Endostar, a modified recombinant human endostatin, suppresses angiogenesis through inhibition of Wnt/beta-catenin signaling pathway[J]. PLoS One, 2014, 9(9): e107463.
[21] Xiao L, Yang S, Hao J, et al. Endostar attenuates melanoma tumor growth via its interruption of b-FGF mediated angiogenesis[J]. Cancer Lett, 2015, 359(1): 148-154.
[22] Yu D, Wu H, Yang B, et al. Antitumor effects of Endostar on non-Hodgkin's lymphoma by regulating endothelial progenitor cells through protein kinase B-dependent pathway[J]. Acta Biochim Biophys Sin (Shanghai), 2013, 45(9):742-748.
[23] Jin F, Ji H, Jia C, et al. Synergistic antitumor effects of endostar in combination with oxaliplatin via inhibition of HIF and CXCR4 in the colorectal cell line SW1116[J]. PLoS One, 2012, 7(10): e47161.
[24] Zhang L, Ge W, Hu K, et al. Endostar down-regulates HIF-1 and VEGF expression and enhances the radioresponse to human lung adenocarcinoma cancer cells[J]. Mol Biol Rep, 2012, 39(1): 89-95.
[25] Wu Y, Zheng Y, Shen Z, et al. Endostar combined with radiotherapy increases radiation sensitivity by decreasing the expression of TGF-beta1, HIF-1alpha and bFGF[J]. Exp Ther Med, 2014, 7(4): 911-916.
[26] Wen QL, Meng MB, Yang B, et al. Endostar, a recombined humanized endostatin, enhances the radioresponse for human nasopharyngeal carcinoma and human lung adenocarcinoma xenografts in mice[J]. Cancer Sci, 2009, 100(8): 1510-1519.
[27] You ZY, Zhao Y, Liu F, et al. The radiosensitization effects of Endostar on human lung squamous cancer cells H-520[J]. Cancer Cell Int, 2010, 10:17.
[28] Fu W, Zhuo J, Hu L. Differential effects of recombinant human endostatin treatment on differentiated and undifferentiated blood vessels in Lewis lung cancer[J]. Oncol Lett, 2017, 13(1): 196-200.
[29] Jain R K. Normalization of tumor vasculature: an emerging concept in antiangiogenic therapy[J]. Science, 2005, 307(5706):58-62.
[30] Jain RK. Normalizing tumor vasculature with anti-angiogenic therapy: a new paradigm for combination therapy[J]. Nat Med, 2001, 7(9): 987-9.
[31] Fukumura D, Jain RK. Tumor microvasculature and microenvironment: targets for anti-angiogenesis and normalization[J]. Microvasc Res, 2007, 74(2-3):72-84.
[32] Stylianopoulos T, Jain R K. Combining two strategies to improve perfusion and drug delivery in solid tumors[J]. Proc Natl Acad Sci U S A, 2013, 110(46): 18632-18637.
[33] Goel S, Duda D G, Xu L, et al. Normalization of the vasculature for treatment of cancer and other diseases[J]. Physiol Rev, 2011, 91(3):1071-121.
[34] Batchelor TT, Sorensen AG, Di Tomaso E, et al.

AZD2171, a pan-VEGF receptor tyrosine kinase inhibitor, normalizes tumor vasculature and alleviates edema in glioblastoma patients[J]. Cancer Cell, 2007, 11(1):83-95.
[35] Hormigo A, Gutin P H, Rafii S. Tracking normalization of brain tumor vasculature by magnetic imaging and proangiogenic biomarkers[J]. Cancer Cell, 2007, 11(1):6-8.
[36] Ning T, Jiang M, Peng Q, et al. Low-dose endostatin normalizes the structure and function of tumor vasculature and improves the delivery and anti-tumor efficacy of cytotoxic drugs in a lung cancer xenograft murine model[J]. Thorac Cancer, 2012, 3(3):229-238.
[37] Rubatt JM, Darcy KM, Hutson A, et al. Independent prognostic relevance of microvessel density in advanced epithelial ovarian cancer and associations between CD31, CD105, p53 status, and angiogenic marker expression: A Gynecologic Oncology Group study[J]. Gynecol Oncol, 2009, 112(3):469-474.
[38] Park JS, Kim IK, Han S, et al. Normalization of tumor vessels by tie2 activation and ang2 inhibition enhances drug delivery and produces a favorable tumor microenvironment[J]. Cancer Cell, 2016, 30(6).
[39] Xin G, Du J, Zhu L, et al. Differential anti-tumor effects for various regimens of endostar plus cisplatin in ovarian cancer[J]. Zhonghua Yi Xue Za Zhi, 2011, 91(47): 3367-3370.
[40] Zhang YY, Sun CF, Peng J. Expression of CD31, CD34 and CD105 in squamous cell carcinoma of tongue and their relationships with lymph node metastasis[J]. Hua Xi Kou Qiang Yi Xue Za Zhi, 2008, 26(2): 201-205.
[41] Fu Y, Tang H, Huang Y, et al. Unraveling the mysteries of endostatin[J]. IUBMB Life, 2009, 61(6): 613-626.
[42] He L, Zhao C, Li Y, et al. Antiangiogenic effects of recombinant human endostatin in lung cancers[J]. Mol Med Rep, 2018, 17(1):79-86.
[43] Huang G, Chen L. Recombinant human endostatin improves anti-tumor efficacy of paclitaxel by normalizing tumor vasculature in Lewis lung carcinoma[J]. J Cancer Res Clin Oncol, 2010, 136(8):1201-1211.
[44] Li N, Zheng D, Wei X, et al. Effects of recombinant human endostatin and its synergy with cisplatin on circulating endothelial cells and tumor vascular normalization in A549 xenograft murine model[J]. J Cancer Res Clin Oncol, 2012, 138(7):1131-44.
[45] Yu M, Han Y, Zhuo H, et al. Endostar, a modified endostatin induces vascular normalization to improve chemotherapy efficacy through suppression of src signaling pathway[J]. Cancer Biother Radiopharm, 2018, 33(4):131-138.
[46] Xu Q, Gu J, Lv Y, et al. Angiogenesis for tumor vascular normalization of Endostar on hepatoma 22 tumor-bearing mice is involved in the immune response[J]. Oncol Lett, 2018, 15(3):3437-3446.
[47] Zheng YF, Ge W, Xu HL, et al. Endostar enhances the antitumor effects of radiation by affecting energy metabolism and alleviating the tumor microenvironment in a Lewis lung carcinoma mouse model[J]. Oncol Lett, 2015, 10(5):3067-3072.
[48] Li W, Zhao X, Du B, et al. Gold nanoparticle-mediated targeted delivery of recombinant human endostatin normalizes tumour vasculature and improves cancer therapy[J]. Sci Rep, 2016, 6:30619.
[49] Jia L, Ren S, Li T, et al. Effects of combined simultaneous and sequential endostar and cisplatin treatment in a mice model of gastric cancer peritoneal metastases[J]. Gastroenterol Res Pract, 2017, 2017:2920384.
[50] Jiang X D, Qiao Y, Dai P, et al. Preliminary clinical study of weekly recombinant human endostatin as a hypoxic tumour cell radiosensitiser combined with radiotherapy in the treatment of NSCLC[J]. Clin Transl Oncol, 2012, 14(6):465-470.
[51] Yuan J, Wu CW, Liu ZJ, et al. Observation of the antitumor effect of endostar combined with docetaxel under different administration sequences[J]. Zhonghua Zhong Liu Za Zhi, 2010, 32(8):580-585.
[52] Jiang XD, Dai P, Qiao Y, et al. Clinical study on the recombinant human endostatin regarding improving the blood perfusion and hypoxia of non-small-cell lung cancer[J]. Clin Transl Oncol, 2012, 14(6):437-443.
[53] Meng MB, Jiang XD, Deng L, et al. Enhanced radioresponse with a novel recombinant human endostatin protein via tumor vasculature remodeling: experimental and clinical evidence[J]. Radiother Oncol, 2013, 106(1): 130-137.
[54] Lv Y, Jiang R, Ma C, et al. Clinical observation of recombinant human vascular endostatin durative transfusion combined with window period arterial infusion chemotherapy in the treatment of advanced lung squamous carcinoma[J]. Zhongguo Fei Ai Za Zhi, 2015, 18(8): 500-504.

